Use of Some Essential Oils as Antimicrobial Agents to Control Pathogenic Bacteria in Beef Burger

M. Alsaiqali, 1,2 A.A. El-Shibiny, 1 M. Adel, 1 M.A.S. Abdel-Samie and 1 S. Ghoneim

1Department of Food and Dairy Sciences and Technology, Faculty of Environmental Agricultural Sciences, Arish University, North Sinai, Egypt
2University of Science and Technology, Zewail City of Science and Technology

Abstract: Meat and meat products such as beef burger are subject to spoilage either by microbes or by fat oxidation. Accordingly, it is very important to protect them from spoilage by adding preservatives, especially natural preservatives to extend their shelf life and to improve their characteristics. The aim of this study was to evaluate the antioxidant activity and antibacterial effect of three different essential oils (thyme, cumin and parsley) at three different concentrations (0.3, 0.6 and 1.2 %) against Staphylococcus aureus, Salmonella enterica, Pseudomonas aeruginosa and Escherichia coli artificially inoculated in beef burger stored at 4±1°C for 4 days. Our results indicated that the high concentration (1.2 %) of essential oils reduced the values of pH, thiobarbituric acid (TBA) and total volatile nitrogen (TVN) in comparison to the control samples. The results of antimicrobial effects of those essential oils were variable. It has been observed that beef burger treated with parsley and thyme oil showed a better reduction effect than cumin on the bacterial count during storage at refrigeration temperature. The high concentrations of essential oils (0.6 and 1.2 %) had the highest inhibitory effect in comparison to the low concentration (0.3 %) with most bacteria during the first period of cold storage. The lowest inhibitory effect was observed with P. aeruginosa. These observations demonstrated that essential oils exhibited variable antibacterial activity against tested bacterial strains. In conclusion, the inhibitory effect of used essential oils could affect positively the quality of beef burger and extends its shelf-life.

Key words: Beef burger · Essential oils · Antioxidant and antibacterial properties · Pathogenic bacteria

INTRODUCTION

Meat and its products are highly perishable and can spoil very easily if they are not store properly. Once they spoiled, they become unhealthy to eat due to microbial growth and chemical changes by enzymes [1]. In general, lipid oxidation is considered one of the most important causes of quality deterioration in meat and it produces the off flavors and odors, in addition to the increase of the drip losses, loss of pigment and as a result decrease the consumer acceptability [2]. This deterioration is mainly due to the contamination of meat and its products during production, handling and consumption [3]. In addition to the cross contamination during processing, poor personal hygiene and inappropriate holding temperature also contribute to the contamination of ready to eat foods [4]. Because meat is very rich with protein, it is highly susceptible to microbial growth, which can cause its spoilage and contributes to food borne diseases in human, resulting in serious health problems [5]. The most important pathogenic bacteria associated with meat products are Salmonella spp., Staphylococcus aureus, Listeria monocytogenes, Clostridium perfringens, Clostridium botulinum, Escherichia coli O157:H7, Pseudomonas, Acinetobacter, Enterobacter, Lactobacillus spp., Leuconostoc spp. and Proteus spp. [6, 7].

It is expected that the growth of most bacteria in meat can be inhibited or completely stop if meat products are preserved by chilling and freezing [8] which keep the original characteristics of meat products [9]. Other methods such as the addition of sodium nitrite, potassium nitrite, sodium nitrate and potassium nitrate can be used to persevere the cured meat and poultry [10]. Sodium
nitrite preserves the unique colors, textures and flavors of meat and poultry products which cannot be maintained by any other additives [11]. However, such chemical compounds can have bad effects on human health [12]. Meat preservation methods became a necessary to transport meat for long distances without spoiling and loss of nutritional value [13].

Essential oils and their extracts can be used as natural additives to reduce the use of chemical preservatives and reduce their risks. Such materials can extend the shelf life of meat and their products and control/inhibit the microbial growth [14]. Many of the essential oils have potential benefits in food production since they showed antibacterial, antifungal and antioxidant effects [15]. The essential oils are good sources of natural antioxidants such as phenolic, flavonoids, alkaloids, tannins and phenolic acids [16] and the TBA value is routinely used as an index of lipid oxidation in meat products [17]. The essential oils have different compounds with antibacterial activities such as; geraniol, menthol, cinnamyl alcohol, linalool, citronellol, carvacrol, cinnamaldehyde, eugenol, thymol, estragole, carvone and chavicol [18]. Different essential oils of different plants showed an effective antimicrobial effects on bacterial count when they are applied in meat products during storage such as; coriander [19], ginger and basil oil [20], rosemary, sage and thyme [21], grape seed extract and pine bark extract [22] and garlic and lemon grass oils [23].

Other plants and seeds such as cumin (Cuminum cyminum) are very rich with natural compounds such as acuminumdehydro, limonene and linalool that have both antimicrobial and antioxidant activity [24-26]. The extracted essential oils of cumin showed an antimicrobial effect against E. coli, Salmonella, Pseudomonas aeruginosa, Staphylococcus aureus and L. monocytogenes [27-30].

Thyme (Thymus Vulgaris) is also a good source of phenolic compounds as g-terpinene, 1, 8-cineole, thymol and eugenol that have antimicrobial effect on pathogenic bacteria [31]. The essential oils of thyme successfully inhibited the growth of E. coli, Salmonella, Pseudomonas aeruginosa and Staphylococcus aureus [32-35]. Thyme oil has also an excellent antioxidant activity when it is added to minced beef during storage and its high value decreases the values of the pH, TVN and TBA [23].

Parsley (Petroselinum crispum) is another herb which is very rich in vitamins, thiamin, carotene, organic minerals and phenolic compounds such as Cosmene, Limonene, Myristicin, α-Pinene and β-Pinene [36 & 37]. In addition to those organic compounds, parsley contains other compounds such as plus oleic, linoleic, palmitic and other fatty acids [38] that showed antimicrobial effects against Salmonella, E. coli and Staphylococcus aureus [37, 39 & 40].

In this study we investigated the possible antimicrobial and antioxidant effects of essential oils as natural preservatives in beef burger during storage at 4 °C. Chemical properties such as pH-value, TBA -value and TVN-value in addition to the inhibitory effects of those oils on the growth of bacterial pathogens such as S. aureus, S. enterica, E. coli and P. aeruginosa in raw beef burger were investigated during storage at 4±1 °C for 5 days.

**MATERIALS AND METHODS**

**Extraction of Essential Oils:** The extraction of thyme, cumin and parsley essential oils was achieved by the hydro-distillation method proposed by Busatta et al. [41]. The dried leaves and seeds of the plants (100 g) were hydro-distilled for 3 h using a Clevenger type apparatus. Then, the essential oil was dried with anhydrous sodium sulfate and kept in sealed vials at 4°C. The extracted oils from thyme, cumin and parsley were added to beef burger at three different concentrations; 0.3, 0.6 and 1.2 % (v/w).

**Preparation of Beef Burger:** About 21.8 Kg of beef burger (Minced meat 70 %, fat 15 %, starch 3.5 %, cold water 10 % and salt 1.5 %) are directly transported to the laboratory in an ice box. All burger pieces were divided to 5 different groups. One group was left without any bacterial inoculation while the other 4 groups were artificially inoculated with 4 different bacterial strains (Staphylococcus aureus ATCC 6538, Salmonella enterica ATCC 25566, E. coli ATCC 8739 and Pseudomonas aeruginosa ATCC 10145). Each of those 5 groups was divided into treated and untreated groups (control). The treated groups were treated with 0.3, 0.6 and 1.2 % (v/w) concentrations of each essential oil (thyme, cumin and parsley). Each burger piece (~35 g) was formed and placed on plastic foam meat trays, wrapped with polyethylene film and stored for 4 days at 4°C.

**Chemical Examination:** Chemical analyses were performed according to standardized methods. Ten grams of beef samples were homogenized with 100 mL distilled water for 1 min. The mixture was used to measure the pH value at room temperature by using pH Suntex meter [42]. Total volatile nitrogen (TVN) was measured according to the technique recommended by FAO [43]. Thiobarbituric
Statistical Analysis: Statistical analyses were performed using SPSS V.15.0 for Windows. ANOVA was carried out on data of the chemical and microbiological evaluations. Data are expressed as mean ± SE [51].

RESULTS AND DISCUSSION

The effect of essential oils on the chemical properties of burger after four days of cold storage was investigated. The pH, TBA and TVN values were determined in both treated and untreated (control) samples according to the previously described method. Burger samples treated with high concentration (1.2 %) of thyme, cumin and parsley oil showed that, the values of pH were significantly low (P < 0.05) compare to control samples. The pH value of control was 6.56 at the beginning of the experiment (Day 0) and has increased to 6.67 after 4 days of storage at refrigeration temperature.

The treated samples with 1.2 % of thyme, cumin and parsley oils reduced the value of pH to 6.58, 6.59 and 6.63 respectively compare to the control samples after 4 days of storage (Figure 1). The highest reduction rate was observed with both thyme and cumin oil treatments. The increased pH value of control beef samples might be due to the effect of microbial growth which may cause protein hydrolysis and release of nitrogenous compounds that increase the pH value of meat [52]. The low values of pH in samples treated with the 3 essential oils after four days of cold storage might be due to the antimicrobial effect of phenolic compound in herb oils. Similar results have been reported before by Salem et al. [23] who stated that, minced beef treated with 1.0 and 1.5 % of thyme, garlic and lemon grass oils showed lower pH values in comparison with untreated samples (control) after 6 days of cold storage. The reduction effect of essential oils such as clove oil on pH values in treated meat after storage at cold temperature have been reported by other researchers [53].

The results presented in Figure 2 showed that the means of TVN values of treated samples with 1.2 % thyme, cumin and parsley oils were significantly low in comparison to the untreated samples (control) after four days of cold storage. The highest reduction rate was observed with thyme and this significant decrease in TVN values in treated samples might be attributed to antimicrobial effect of those essential oils and to their ability to inhibit the activity of proteolytic enzymes [54].
Our findings are quite similar to the published results by Salem et al. [23] who stated that, the use of thyme, garlic and lemon grass oils successfully decreased the rate of TVN in treated beef in comparison to the control samples after six days of cold storage. Other studies have indicated that essential oils such as thyme, rosemary, marjoram oil and ginger significantly ($p < 0.05$) decreased the value of TVN in treated minced meat stored at cold temperature [55, 56].

The highest value of TBA was observed in control samples (0.842 mg malonaldehyde/ kg) after 4 days of storage at refrigeration temperature in comparison to the treated samples with essential oils. Thyme essential oil reduced the value of TBA significantly ($P < 0.05$) in comparison to control samples. The reduced rate of TBA values in treated samples might be due to the preservative effect of essential oils which slowed down or reduced the autolysis in meat muscles and accordingly reduced the
breakdown of protein. The activity of used essential oils is due to their content of phenolic and flavonoid compounds [57]. The high value of TBA in control samples may be attributed to the auto oxidation of meat lipids in addition to the bacteriological and oxidative rancidity [23]. Our results are similar to those published by Ashour et al. [58] and Kassem et al. [59] who reported low values of TBA in treated meat with 0.05 % thyme oil after six days of storage at 4 °C. In another study, Sharafati-Chaleshtori et al. [60] did not observe any significant reduction in TBA values after adding different concentrations of basil essential oil to raw beef burger during storage 4 °C for 12 days.

Table 1 summarizes the values of MIC of the tested essential oils against the selected bacterial strains. The results demonstrated that all three essential oils displayed an antimicrobial activity with low and minimum concentration against both S. enterica and E. coli while higher concentrations are needed (> 1.2 %) to inhibit the growth of both S. aureus and P. aeruginosa.
Different concentrations (0.3, 0.6 and 1.2 %) of the essential oils have been used to study their effect to control or inhibit the growth of different pathogenic bacteria in beef burger. Figure 4 presents the effect of different concentrations of parsley oil on the growth rate of TBC, S. enterica, E. coli, S. aureus and P. aeruginosa during storage at refrigeration temperature for 4 days. The results indicated that, parsley oil had a great reduction effect on the numbers of TBC, S. enterica, E. coli, S. aureus and P. aeruginosa during the first 2 days of the experiment. However, this inhibitory effect has been decreased by day 2 except with P. aeruginosa. Up to 2.5 log 10 CFU/ml reduction rate has been observed with E. coli with the high concentration treatment (1.2 %) after 1 day of the experiment while the lowest reduction rate was observed with P. aeruginosa after 1 day of treatment. The other 2 lower concentrations (0.3 and 0.6 %) had less inhibitory effect in comparison to the high concentration. After 2 days of refrigeration storage, we noticed less effect and sometimes no effect of 0.3 % of parsley concentration on the growth of most bacteria especially S. aureus. The primary observed reduction of bacterial counts may be attributed to the sensitivity of pathogens to the cold storage temperature in addition to the antimicrobial effect of different compounds in parsley. Similar results have been reported by other researchers who observed an antimicrobial effect of parsley oil on E. coli [37, 38], E. coli O157:H7 and Micrococcus in a model food system [61] and E. coli and Klebsiella [39].
The antimicrobial effect of parsley oil when used with high concentration also has reported against *Pseudomonas* spp. [37, 40]. Some other essential oils such as oregano showed high reduction rate against *Pseudomonas* in meat samples during refrigerated storage [62]. Green tea was also reported to have a significant antibacterial activity against *Pseudomonas aeruginosa* [63]. The inhibitory effect of parsley may be attributed to the presence of phenolic compounds as indicated before [40, 64].

The inhibitory effect of cumin oil on TBC, *S. enterica*, *E. coli*, *S. aureus* and *P. aeruginosa* in beef burger is presented in Figure 5. Cumin oil had a similar inhibitory effect like parsley and the highest reduction rate (2.5 log$_{10}$ CFU/ ml) was observed with TBC followed by *S. enterica* (2.3 log$_{10}$ CFU/ ml) after 1 day of treatment. The lowest inhibitory effect has been observed with both *E. coli* and *P. aeruginosa* (Up to 1 log$_{10}$ CFU/ ml reduction with *E. coli*). Generally, all cumin oil concentrations showed a reduction effect on bacteria. However, there is no significant difference between 0.6 % and 1.2 % concentrations with most bacteria. The reduction rate observed with cumin oil with both TBC and *S. enterica* was greater than that observed with parsley oil during the first 2 days of treatment. Our results are quite similar to the results reported by Rasool [65] and Wanner *et al.* [66] who recorded antimicrobial properties of four different spices including cumin on *E. coli* in meat products.
The antimicrobial effect of cumin oil has been observed before against S. aureus [28 & 67] and against P. aeruginosa [68]. Different concentrations of cumin showed inhibition effect against S. enterica in beef burger during cold storage [60] and its effect as a preservative is well known before due to its antioxidant properties [69 & 70] in addition to its ability to enhance the flavor of foods [71].

Data presented in Figure 6 showed that the high concentration (1.2 %) of thyme oil had a significant (p < 0.05) reduction on TBC, S. enterica and E. coli after 2 days of treatment and storage at refrigeration temperature. The maximum reduction rate (2.5 log_{10} CFU/ml) was observed with S. enterica. The high concentration (1.2 %) of the thyme oil had the highest inhibitory effect in comparison to the other two concentrations (0.3 and 0.6 %). The antimicrobial effect of thyme oil has been observed in a previous study. Solomakos et al. [33] reported that different concentrations (0.3, 0.6 or 0.9 %) of thyme oils were very effective in reducing the numbers of E. coli O157:H7 in treated minced beef. In addition, the addition of thyme and marjoram oils decreased the numbers of E. coli in minced pork after 24 h of storage at 5 °C [72]. In another study, the addition of 0.5 % thyme oil to chicken breast meat reduced the numbers of E. coli O157:H7 significantly [73].

The lowest inhibitory effect has observed with P. aeruginosa. However, previous results indicated that thyme oil was able to reduce the numbers of Pseudomonas in lamb meat and beef burger during cold storage [34, 74]. Other previous studies showed the effectiveness of different essential oil to reduce the numbers of Pseudomonas in different product including chicken breast meat samples [27, 75, 76]. The observed high effect of thyme to reduce the numbers of S. aureus has been reported before in beef burger [59] and minced beef during storage at 4°C [23,33]. Previous works showed similar results about the effect of herb oils such as oregano to reduce the numbers of S. enterica and E. coli O157:H7 in chicken breast meat after storage at 4 °C for 72 h [77] and Listeria in meat pieces [78].

In general, essential oils can be used to reduce the use of chemical preservatives and to produce safe food due to their antioxidant, antifungal and antibacterial activity. Essential oils can be used to prevent foodborne diseases and pathogenic bacteria to improve the quality of meat characteristics due to their aroma and also to extend shelf-life of its products. It has been observed that beef treated with parsley and thyme oil showed a better reduction effect than cumin on the bacterial count during storage at refrigeration temperature. The high concentrations of essential oils (0.6 and 1.2 %) had the highest inhibitory effect in comparison to the low concentration (0.3 %) with most bacteria during the first period of cold storage. Similar results were obtained [19, 73, 79, 80] and Viuda-Martos et al. [57] who reported that thyme, parsley, rosemary and clove and other essential oils had a significant inhibitory effect against several pathogenic and food-spoiling bacteria. For example, Muhammad and Ali [32], Husni et al. [40] and Patil et al. [81] reported that cumin, thyme and parsley oils had a significant inhibitory effect against Salmonella and also against Shigella [60] during cold storage for 9 days. Other essential oils like oregano, thyme and cinnamon [77, 82, 83] showed a good inhibitory effect against Salmonella in chicken breast meat and minced sheep meat.

Antimicrobial activity of different essential oils is directly linked to the presence of phenolic constituents such as eugenol cumin aldehyde, carvone, limonene, linalool and thymol [25, 84, 85]. Most of those active phenolic compounds are able to inhibit the growth of bacteria by targeting the amino acid decarboxylase for example [86]. For example, carvone, carvacrol, cinnamaldehyde and eugenol showed a significant inhibitory effect against S. aureus [87, 88]. The refrigeration temperature may also contribute to the initial reduction rate of the bacterial numbers due to their sensitivity to the cold check. Those active compounds of essential oils may interact with the bacterial cell membrane, causing the leakage of its cellular components and accordingly cause the cell death [89]. They also can inhibit the activity of enzymes involved in the energy regulation and metabolism [31]. The reduced inhibitory activity of essential oils added to meat may be attributed to the presence of fats and other components in meat [31].

CONCLUSIONS

In conclusion, some essential oil tested in this study could be used as natural preservatives to extend the shelf-life of beef burger and to minimize lipid oxidation. Some oils showed minimum to moderate antimicrobial effect, thus they rather could be used with other essential oils to control bacterial infection in meat products. Further studies are required to study the active compounds of these essential oils.
REFERENCES


